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# Monitoring for potential residual disease activity by serum insulin-like growth factor 1 and soluble Klotho in patients with acromegaly after pituitary surgery: Is there an impact of the genomic deletion of exon 3 in the growth hormone receptor (d3-GHR) gene on "safe" GH cut-off values?

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ABSTRACT

*Background:* Acromegaly is an illness usually defined by excessively high growth hormone (GH) and insulin like growth factor 1 (IGF-1) levels, the latter mainly reflecting GH action on the liver. IGF-1, also known as somatomedin C, mediates several actions of GH. The diagnosis and management of acromegaly is relatively straight forward, but long-term follow-up of patients can be difficult, as elevated IGF-1 levels can occur in the presence of apparently normalised GH levels and late recurrence of acromegaly may arise despite previous suppression on oral glucose tolerance testing. Data suggest this applies especially to patients in whom the GH receptor lacks exon 3. In such patients, GH may not always be a useful marker of disease, and traditional GH cut-offs may be misleading. Recent data suggest that soluble Klotho (sKlotho), besides and in addition to IGF-1, may help monitor the activity of GH-producing adenomas (presumably reflecting GH action on the kidneys) and may be a useful supplementary tool.

*Methods:* GHR genotyping was performed in 112 patients with acromegaly. IGF-1 and sKlotho levels were measured in the sera of patients before and after transsphenoidal surgery, with emphasis on patients judged inconclusively cured by surgery or with small residual tumour masses shortly after surgery. Patients were assessed for recurrence of acromegaly with GH levels (random or nadir during an oGTT).

*Results:* Of the 48 patients who underwent surgery between 2000 and 2009 and who had well-documented longer term follow-up at our institution, 29 had no biochemical evidence of residual disease activity after transsphenoidal surgery (marked reduction in IGF-1 and sKlotho levels, GH suppressible to <1 ng/ml) and were classified as in remission. 2 of these patients developed recurrent symptoms of acromegaly during follow-up with increasing levels of IGF-1 and sKlotho, and both patients were carriers of the d3-GHR genotype.

*Conclusions:* Acromegalic patients with the d3-GHR polymorphism might be – for a given low postsurgical GH level – at higher risk for recurrence and may require a lower GH nadir during oGTT to be classified as in remission. Soluble Klotho could be useful in the follow-up of acromegalic patients. The question arises whether sKlotho not only reflects the activity of GH-secreting pituitary adenomas but whether Klotho (ectodomain clipping?) could also mediate selected actions of GH.



## 1. Introduction

The growth hormone receptor (GHR) is expressed in many tissues and mediates the effect of growth hormone (GH), which is of great importance for skeletal growth and the attainment of adult height. GH secretion by pituitary somatotropic cells is mainly regulated by hypothalamic neuro-endocrine mediators such as

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somatostatin and GHRH, but also by signals from the periphery such as ghrelin and by feedback inhibition via insulin like growth factor 1 (IGF-1). There has been a lot of interest in the GHR genotype in recent years, after it was found that there are two main isoforms of GHR in humans, a full-length isoform (fl-GHR) and an isoform that lacks exon 3 (d3-GHR). This species-specific expression pattern is due to two distinct GHR transcripts, characterized by retention or exclusion of exon 3, respectively (Pantel et al., 2000). The d3-GHR polymorphism occurs in the heterozygous state in 30–40% and in the homozygous state in 10–20% of the population, while the remaining half of the population is homozygous for the fl isoform (Dos Santos et al., 2004). Exon 3 encodes for a part of the extracellular, ligand-binding domain of the GHR. Although wild type fl-GHR and mutant d3-GHR have comparable *in vitro* GH-binding properties, d3-GHR displays a greater response to GH stimulation. At any given GH level, enhanced intracellular signal transduction with increased transcriptional activity on target genes has been observed, with the transduction of GH signalling being approximately 30% higher in both d3-GHR homodimers and d3/fl-GHR heterodimers than in fl-GHR homodimers (Dos Santos et al., 2004). The significance of the d3-GHR in patients with growth hormone disorders remains unclear and is of particular interest.

The *klotho* gene was discovered in a mouse model in 1997 after its accidental disruption (by insertion of ectopic DNA) caused a phenotype of accelerated aging (Kuro-o et al., 1997). Later, it was found that aging suppression and lifespan extension could be achieved in mice by overexpression of Klotho (Kurosu et al., 2005). Klotho is the goddess of fate, 'spinning the thread of life' in Greek mythology. Mice with deficient Klotho expression have a shorter life span and premature skin atrophy, osteoporosis, hypogonadotropic hypogonadism, arteriosclerosis, pulmonary emphysema and neuro-degenerative disorders. The *klotho* gene encodes a single-pass transmembrane protein (1014 amino acids) with a short cytoplasmic domain (10 amino acids) and an extracellular domain composed of two beta-glycosidase-like tandem repeats (KL1, KL2) and is predominantly expressed in kidneys, choroid plexus and several endocrine organs including the pituitary, the parathyroids, the pancreas, and reproductive organs (gonads, placenta) (Kuro-o et al., 1997; Li et al., 2004). The function of the Klotho protein remained an enigma for years but in 2006, two independent groups reported that membrane Klotho served as a co-receptor for fibroblast growth factor 23 (FGF23, a hormone produced by osteocytes which inhibits renal phosphate reabsorption and calcitriol production) and thereby plays an important role in phosphate homeostasis (Kurosu et al., 2006; Urakawa et al., 2006). Klotho protein also exists in a soluble form which can arise either from a distinct transcript or from secretase-catalyzed ectodomain shedding of membrane Klotho (Imura et al., 2004; Matsumura et al., 1998). It is released into the extracellular space and soluble Klotho (sKlotho) can reach and affect a number of target tissues and processes, including regulation of hormone and growth factor (e.g. inhibition of insulin/IGF-1) signalling, and of ion channel and transporter plasma membrane abundance and activity, e.g. to attenuate calciuria. Currently, sKlotho should not be labelled as a hormone since cognate receptors have not been identified thus far. However, circulating sKlotho can regulate biological processes, e.g. by (enzymatic) glycan modification. Illustrating an action on the nephron, sKlotho prevents endocytosis of transient receptor potential cation channel, subfamily V, member 5 (TRPV5) (Chang et al., 2005). The increase in retention time on the plasma membrane enhances ion channel activity and thereby decreases renal calcium loss (Alexander et al., 2009; Asai et al., 2012). The soluble form which is released into the circulation can be detected by an ELISA (Yamazaki et al., 2010). Levels of sKlotho decrease with age in healthy subjects and are inversely related with mortality in the elderly (Yamazaki et al., 2010; Semba et al., 2011). The kidneys appear to be the major source of sKlotho in sera of human subjects; accordingly, serum sKlotho decreased by about 40% following nephrectomy in living donors (Akimoto et al., 2013). Although renal expression of the *Klotho* gene and urinary Klotho levels have been found to be decreased in renal disease, it currently remains controversial whether plasma sKlotho is a useful biomarker for kidney disease (Asai et al., 2012; Koh et al., 2001; Akimoto et al., 2012; Hu et al., 2011).

Acromegaly leads to high serum phosphate and FGF23 levels despite enhanced GFR, as well as to insulin resistance and hyperglycaemia (Ito et al., 2007). As Klotho levels may relate to FGF23 and insulin resistance (or high phosphate and glucose, respectively) in acromegaly, it is of particular interest in these patients, and we therefore measured sKlotho in sera of patients before and after transsphenoidal surgery.

## 2. Effect of the d3-GHR in healthy subjects

It is important to note that as long as normal GH secretion and an intact feedback mechanism are present, potential variations in GH sensitivity due to GHR differences can be compensated by endogenous pituitary GH secretion. The extracellular fragment of the GHR is cleaved from the cell surface and circulates as a GH binding protein (GHBP). GHBP prolongs the plasma half-life of circulating GH. Wan et al. recently reported that the GHR exon 3 polymorphism could be assessed not only by DNA analysis but also by protein analysis of serum samples using an ELISA measuring total and exon 3-positive GHBP. Subjects with the fl/fl genotype (all GHBP molecules exon 3-positive) had higher levels of total GHBP in serum, followed by those with the d3/fl genotype, whereas those with the d3/d3 genotype (all GHBP molecules exon 3-negative) had the lowest total GHBP levels (Wan et al., 2013). It has, however, not been formally tested whether the GHR isoform has an influence on (GHR-mediated?) clearance of GH from the circulation.

## 3. Effect of the d3-GHR in a pediatric population with growth deficiency

In children with GH deficiency, replacement therapy is a widely accepted and efficient treatment option. As the d3-GHR polymorphism could affect the response to GH therapy, various subgroups of children with growth deficiency have been investigated. In their original work, Dos Santos and co-authors found that children carrying an allele encoding the d3-GHR variant were more responsive (in terms of growth) to GH administration than children homozygous (fl/fl) for the wild type GHR (Dos Santos et al., 2004). These findings were confirmed by some studies but were challenged by others, who found no impact of the d3-GHR allele on the response to GH treatment and concluded that the effect of the d3-GHR allele on responsiveness to GH therapy is controversial (Blum et al., 2006).

As mentioned above, variations in sensitivity to GH may be irrelevant as long as compensation by endogenous GH secretion works well. This should be kept in mind when discussing several studies on GH therapy to stimulate growth where an influence of the GHR genotype might be expected, especially when low doses of GH are given to patients with severe GH deficiency. These findings generally have limited consequences in clinical practice as GH doses during treatment are adjusted according to their effects on IGF-1 and especially the growth of a child (and not according to the GHR polymorphism). Therefore, a recent editorial concluded that GHR genotyping has a limited impact in clinical medicine (Bougneres, 2010).

## 4. Effect of the d3-GHR on clinical and biochemical parameters in acromegaly

The diagnosis of acromegaly is generally straightforward, as long as clinicians are aware of this possibility. Patients have typical signs and symptoms and elevated GH and IGF-1, the marker and mediator of GH actions, on blood testing. The diagnosis is confirmed by non-suppressible GH levels (nadir >1 ng/ml) during oral glucose tolerance testing (oGTT). The distribution of the GHR isoforms in various acromegaly cohorts has been found to be comparable to that seen in the general population. Consistent with the finding that the d3-GHR shows enhanced GH signal transduction, acromegalic patients carrying (at least one) d3-GHR allele, on average, appear to require only half the amount of GH to cause the same increase in IGF-1 and to have a given severity (signs and symptoms) of the disease when they present for diagnosis as do carriers of the fl-GHR isoform (Schmid et al., 2007). However, conflicting data exist with regard to the impact of the d3-GHR mutation on disease severity. In some study populations, the d3-GHR polymorphism was associated with younger age at diagnosis or more severe symptoms and more complications (e.g. osteoarthritis, colonic polyps and dolichocolon and possibly type 2 diabetes (Kamenicky et al., 2009; Wassenaar et al., 2009). When the diagnosis of acromegaly is made, knowledge of the GHR genotype is not expected to influence therapeutic decisions. However, a recent study by Bernabeu et al. suggested that the d3-GHR carrier status could predict a better response to pegvisomant, findings which have been challenged by others (Bernabeu et al., 2010; Filopanti et al., 2012).

## 5. Relevance of the d3-GHR during follow-up of patients with acromegaly

The main difficulty in the care of acromegalic patients is long term follow-up, as residual disease activity needs to be recognised and treated. Imaging with MRI is inferior to biochemical testing. As GH-producing tumours grow slowly and postoperative radiological changes in the pituitary hamper the interpretation of image, MRI is able to visualize minor mass increases in only a small number of patients (Zirkzee et al., 2004). Therefore, to rule out or to rule in tiny tumour remnants, biochemistry is considered more sensitive than imaging.

The difficulty with biochemical follow-up is that there can be discrepancies between GH and IGF-1, the classical biochemical markers of acromegaly. Overall, GH function tests have a better discriminating readout but IGF-1 appears to be more closely related to disease activity and correlates more closely with morbidity and excess mortality in acromegaly both are important (Freda, 2009). On theoretical grounds, assessing GH activity (in part reflected by IGF-1) appears to be more relevant than merely assessing GH levels. Ideally, IGF-1 should normalise and glucose-suppressed GH should be low after surgery for acromegaly. Most often, 1 ng/ml has been used as a cut-off value (Giustina et al., 2010). However, this ideal scenario is often not clinical reality, with 9–39% of patients showing abnormal GH suppression during oGTT despite a normal IGF-1 level and 24–62% of patients showing elevated IGF-1 levels despite adequate GH suppression (Freda, 2009). Many diseases may cause inadequate GH suppression, such as chronic renal insufficiency, liver disease, malnutrition or hyperglycaemia, to name a few. The GH nadir also seems to be influenced by sex (females having higher values) and body weight, as a negative correlation with BMI has been described (Ronchi et al., 2007). To complicate matters further, oGTT is generally unreliable in patients with diabetes; it should be performed only in patients with well controlled glycaemia, when the glucose challenge can be applied at a time of close to normal baseline glucose so that a glucose rise does occur and is perceived by hypothalamic neurons. IGF-1 levels are rarely above normal in conditions other than acromegaly, but can be elevated in some adolescents. Moreover, measuring IGF-1 remains notoriously difficult, especially since the less time-consuming modern assays do not try to remove IGF binding proteins before immunoassay analysis and interferences are thus more common. In patients with discrepant GH and IGF-1 results, testing is usually repeated.

Recurrent disease activity is probably more often due to the growth of remaining tumour cells than to a de novo formation of another pituitary adenoma. Acromegalic patients with the d3-GHR genotype could be expected to be at higher risk for clinical or biochemical recurrence for a given GH level than those with fl-GHR, because of the enhanced signal transduction transmitted by the d3-isoform. Bianchi et al. have found that around 70% of the patients with elevated IGF-1 levels in the presence of apparently normalised GH levels after surgery carried at least one d3 allele (Bianchi et al., 2009). This suggests that GH may be a less sensitive marker of disease activity in carriers of the d3-GHR polymorphism. Moreover, a different (possibly lower) GH nadir on oGTT may be necessary for such patients, and they probably need an especially careful and long-term follow-up. It is obvious that the majority of studies addressing marginal residual disease activity suffer from confounding by drug treatment efforts to control excessive GH secretion and activity.

## 6. Relevance of sKlotho during follow-up

We determined the levels of sKlotho with a novel ELISA in 24 consecutive, treatment-naïve acromegalic patients referred for their first surgery (2006–2009) to our institution before and 1–3 months after transsphenoidal surgery (Yamazaki et al., 2010). We found that sKlotho levels were markedly increased in relation to GH excess and declined towards normal levels after surgery (Sze et al., 2012). In an additional, prospective study we found that sKlotho was markedly elevated in sera of patients with active acromegaly but not in patients with clinically non-functioning pituitary adenomas, and that sKlotho rapidly decreased towards normal following successful surgical removal of the GH-secreting adenoma (Neidert et al., 2013). These observations suggested that sKlotho might be a GH-dependent serum protein. Overall, until 2012, we assessed serum IGF-1 and sKlotho before and 1–3 months after surgery in a total of 50 patients with newly diagnosed acromegaly seen at our institution over the past decade: IGF-1 levels (mean ± SEM) dropped from 579 ± 32 before to 198 ± 10 ng/ml after surgery, and sKlotho from 4113 ± 415 to 779 ± 63 pg/ml; levels fell in all patients tested. Levels of sKlotho and IGF-1 appeared to be similarly dependent on GH. The mechanisms by which elevated GH leads to excess sKlotho remain unclear. The observation that sKlotho appears to depend on GH-producing adenomas to a comparable extent as IGF-1 raises the possibility of using sKlotho as an additional serum marker in the long term follow-up of acromegalic patients, as sKlotho may reflect the activity of GH-producing tumours.

## 7. Patient examples from the Zurich cohort

Since 2004, we have genotyped 112 patients with acromegaly (after giving written informed consent to participate in this local ethics committee-approved study) at our institution; 44 of them have been reported previously (Schmid et al., 2007). 58 (52%) patients were homozygous for the fl-GHR (wild type), 43 (38%) patients had one d3-GHR allele (heterozygous) and 11 (10%) patients had two d3-GHR alleles (homozygous for the gene

encoding the shorter receptor), a distribution comparable to the normal population.

We focussed on the potential effect of the d3-GHR on our more recent cohort of acromegalic patients, all operated by the same neurosurgeon (R.B) and with histologically proven GH-secreting adenoma (Bellut et al., 2010). Written informed consent, GHR genotyping and follow-up data were available for 48 patients with a diagnosis of acromegaly between 2000 and 2009 (some of whom had recurrent disease). 24 of these patients were homozygous for the fl-GHR, 19 patients had one d3-GHR allele and 5 patients had two d3-GHR alleles. GH (random or nadir during an oGTT) and IGF-1 levels obtained during follow-up were available for analysis, and sKlotho measurements were also performed in the majority of patients in more recent years.

Fig. 1 illustrates the follow-up of four selected individual cases. Overall, there are concomitant and parallel changes in serum IGF-1 and sKlotho over time in a given individual patient, fairly reliably reflecting disease activity; however, in exceptional situations, levels may be discordant, as illustrated by the patient presented as case B. It seems that GH-stimulated (hepatic) IGF-1 production is more strongly attenuated by inflammation, oral oestrogens and pregnancy than GH-stimulated (renal) sKlotho release. Patients A and C were cured following surgery (IGF-1 remained <300 ng/ml and sKlotho <1000 pg/ml during follow-up), whereas patient D suffered from recurrent disease activity (discussed below, in the context of GHR).

*Case A:* This was a 50-year-old female with a long-lasting (in retrospect, 12 years) history of acromegaly. She had an adenoma (1.6 ml, tumour volume estimated as in Bellut et al. (2010)) removed, followed by a marked fall in serum IGF-1 and sKlotho. There was no evidence for pituitary insufficiency, and no evidence for persisting disease activity; GH random values <1 ng/ml were found. Serum levels of IGF-1 (<300 ng/ml) and sKlotho (<1000 pg/ml) remained low during follow-up.

*Case B:* This 28-year-old female presented with a relatively short history of acromegaly and a huge adenoma (27 ml). Only partial surgical debulking was feasible, and she remained with a tumour remnant and active acromegaly. Of note, discordant IGF-1 and sKlotho levels were found in this patient on several occasions: first, she developed bacterial coxitis in the immediate postoperative period; IGF-1 fell transiently into the normal range (to <300 ng/ml) while sKlotho remained high (7900 pg/ml). She was then treated with radiotherapy, followed by sandostatin LAR (SS), and received hormone replacement therapy (including oral oestrogens) for subsequent pituitary failure. There was semi-concordant control of IGF-1 (satisfactory, <300 ng/ml) and sKlotho (partial response, levels at 2000 pg/ml). Later, during gonadotropin-induced pregnancy, SS was paused, resulting in essentially unchanged IGF-1 levels (as anticipated, considering the characteristic spontaneous drop in IGF-1 during pregnancy in patients with acromegaly but in a rise in sKlotho up to $\geqslant$4000 pg/ml (last two points in graph 1B) (Wiesli et al., 2006).

*Case C:* This 37-year-old male patient presented with an adenoma (4.2 ml) which was, in retrospect, only partially removed by the first surgery. A second operation resulted in a further decrease in serum IGF-1 and sKlotho, to disappearance of disease activity and to a GH suppressible to <1 ng/ml on oGTT (Fig. 2A) at the time of writing, 5 years after the patients' first surgery.

*Case D:* This 40-year-old lady had apparently successful removal of an adenoma (2.5 ml) for up to two years after surgery. She carried one d3-GHR allele. Twenty months postoperatively, she suppressed GH from a baseline of 7.16 ng/ml to a nadir of 0.56 ng/ml during oGTT; IGF-1 was within the high-normal range at that time. The shape of the GH curve suggested that normal rather than adenomatous GH-secreting cells contributed to the bulk of GH in the patients' serum. Thirty months postoperatively, IGF-1 and sKlotho began to increase, and on repeat oGTT, the GH nadir was 0.84 ng/ml. 43 months postoperatively, IGF-1 had further increased, and GH suppressed from a baseline of 3.05 ng/ml to a minimum of only 1.02 ng/ml on oGTT testing (Fig. 2B). Thus, according to standard guidelines, she had recurrent acromegaly. An MRI of the pituitary failed to show any evidence of a pituitary tumour. Because the patient had increasing symptoms of acromegaly, treatment with cabergoline was begun 80 months after surgery, resulting in decreasing IGF-1 and sKlotho levels with partial relief of symptoms.

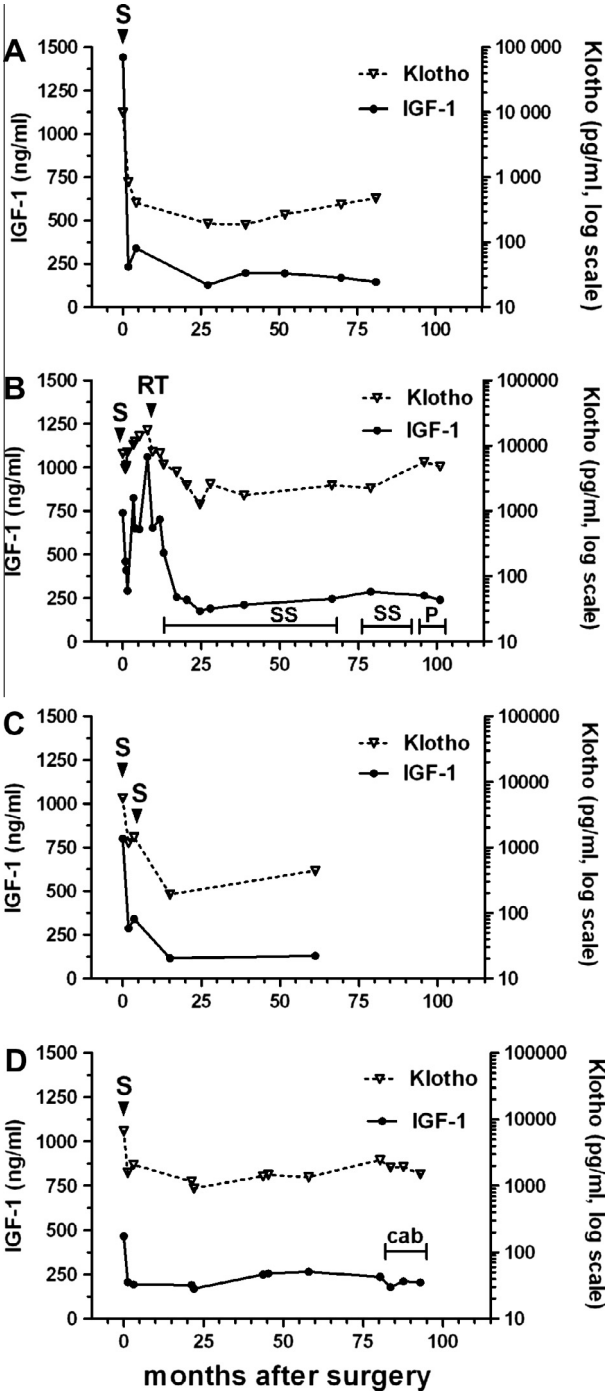


**Fig. 1.** Changes in IGF-1 and sKlotho in four patients over time. Cases A, C and D show a marked concordant reduction in serum IGF-1 and sKlotho after transsphenoidal surgery, Case B illustrates discordant IGF-1 and sKlotho levels during long-term follow-up. A detailed description of the patients is included in the text.

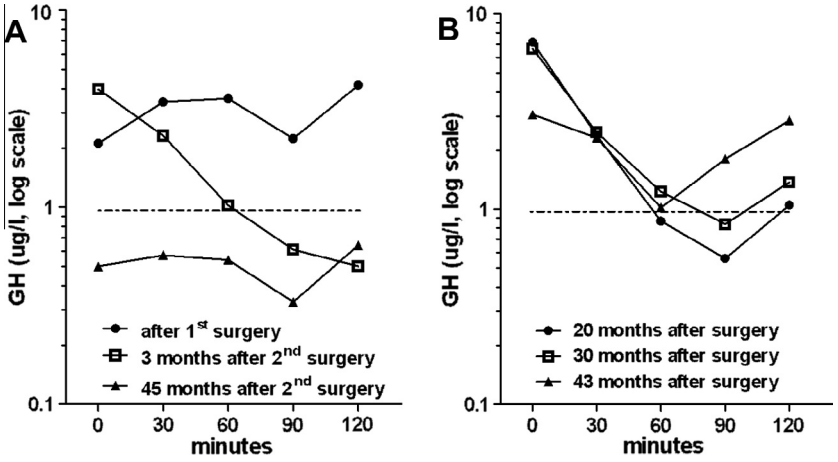


**Fig. 2.** GH suppression during oGTT over time in a patient (A) with residual adenoma and improved GH suppression during oGTT after an additional operation, and follow-up of a patient (B) carrying one d3-GHR and recurrent disease activity.

Among our 48 patients with a diagnosis of acromegaly between 2000 and 2009 and close follow-up, 45 had obvious findings (as judged by four concordant criteria, namely (1) clinical, (2) GH-levels, (3) IGF-1 levels and (4) imaging) in favour of or against persistent disease activity. As stated above, 24 of these 48 patients were homozygous for the fl-GHR, 19 patients had one d3-GHR alleles and 5 patients had two d3-GHR alleles. One of the patients with an obvious residual tumour mass (an adenoma remnant in the cavernous sinus) and persistent disease activity and elevated IGF-1 (values >500 ng/ml) occasionally reached random GH values of <1 ng/ml; he had two d3-GHR alleles. Three of the 48 patients had ambiguous findings during follow-up, despite initial postoperative GH suppression <1 ng/ml and clinical evidence for remission of acromegaly; all 3 of them carried at least one d3-GHR allele.

The 1st patient is described in case D (she was heterozygous for the d3-GHR allele), in her case, a remnant tumour finally became detectable on MRI after 8 years of follow-up (she is currently willing to undergo repeat surgery). Fig. 2B demonstrates the progressive lack of GH suppression during oGTT over time in this patient. Fig. 2A, in comparison, illustrates physiological GH suppression during oGTT after repeated transsphenoidal surgeries in a patient with initially evident residual adenoma (patient shown in panel C of Fig. 1).

The 2nd patient, a 45-year-old man with two d3-GHR alleles, developed recurrent symptoms of acromegaly after 1 year of follow-up. He had no visible residual tumour on MRI postoperatively and GH suppressed to a nadir of 0.2 ng/ml during postoperative oGTT, but IGF-1 gradually increased above the upper limit of normal. He complained of debilitating joint pain, a subsequent pituitary MRI revealed an intrasellar mass suspicious for tumour regrowth (questionable, not confirmed in subsequent imaging).

The 3rd patient, a 52-year-old woman with one d3-GHR allele, suppressed GH on oGTT to 0.52 ng/ml 6 months and to 0.63 ng/ml 31 months after surgery. However, IGF-1 gradually increased and pituitary MRI suggested a remnant adenoma. The patient herself, however, denied recurrent symptoms of acromegaly.

α-Klotho has been found to be a central player in calcium and phosphate homoeostasis (Hu et al., 2013). As compared to intra- and paracrine FGFs, FGF23 as binding partner of a transmembrane α-Klotho-containing complex emerged later during evolution. FGFs are a large family of peptides which, together with their receptor tyrosine kinases (FGFRs) comprise a signalling system that controls development and homoeostasis. The endocrine (FGF19) subfamily (including FGF19, FGF 21 and FGF23) appears to be vertebrate specific and is characterized by a lack of a heparin binding domain so that they escape capturing by the nearby extracellular matrix and reach the circulation (Itoh and Ornitz, 2011). Moreover, endocrine FGFs do not require heparan sulfate proteoglycans for efficient FGFR interaction; however, they need transmembrane klotho(s) as co-receptors instead. Considering evolution, it is obvious that the (later) development of a bony endoskeleton in higher vertebrates and adaptation to terrestrial life set increasingly higher demands and required additional signals (for and from bone) to ensure and regulate energy homeostasis, metabolism, nutrient and ion and mineral handling, and to communicate with intestine (for absorption) and the kidney (for excretion) (Hu et al., 2013). Two *Fgf-like* genes, *egl-17* and *let-756*, and two *Klotho-like* genes, *klo-1* and *klo-2* have been identified in the nematode *Caenorhabditis elegans* (Huang and Stern, 2005; Polanska et al., 2011). In the nematode *C. elegans,* the corresponding KLO-1 and KLO-2 proteins are expressed in the intestine, in the excretory canal and in the hypodermis (Polanska et al., 2011). Unlike the vertebrate Klotho proteins, KLO-1 and KLO-2 in nematodes lack a transmembrane domain. The ancestral "truncated" Klotho forms in nematodes and in *Drosophila* contain only one KL domain whereas the full length transmembrane vertebral Klothos are composed of two KL domains. In men, the alternatively spliced form for the shorter, directly secreted isoform, is reminiscent of these genes (Matsumura et al., 1998)

IGF-1 and sKlotho are both relatively abundant in the circulation; both of them are regulated by GH. In the case of IGF-1, there is fairly good evidence that it mediates some actions of GH and that there is a negative feedback. Indeed, there is pituitary expression in bony fish and in humans, and it may well be that there is not only a negative feedback from circulating (endocrine) IGF-1 but also form locally produced (paracrine or autocrine) IGF-1 (Guler et al., 1989; Eppler et al., 2007; Jevdjovic et al., 2007). In the case of Klotho, there is a lack of data to show whether it mediates actions of GH, and there is no proof for receptors in the pituitary which could mediate a negative feedback. Still, the expression of Klotho has been demonstrated in the pituitary, in the original paper on its discovery at the gene level as well as by immunohistochemistry in the human pituitary (Kuro-o et al., 1997; Neidert et al., 2013).

## 8. Conclusion

Our data, and in particular the 3 patients with increasing IGF-1 levels despite initial postoperative GH suppression <1 ng/ml described above, suggest that individuals with the d3-GHR polymorphism could indeed be at higher risk for recurrence of acromegaly

and have a lower chance of achieving IGF-I normalization after therapy, as suggested previously (Mercado et al., 2008). Although GH levels remained relatively low in the 3 patients, disease activity, as well as IGF-1 and sKlotho levels, increased. Possibly, a lower GH nadir during oGTT needs to be defined for such patients. In our view, GH actions (i.e. clinical symptoms and signs, IGF-1 and sKlotho) are more important than GH levels when estimating the (negative) impact of potential remnant adenomas.

At our institution, we do not use standardised questionnaires (or scores) but rather focus on the severity (over time) of the personal leading symptoms and signs in the particular individual patients we care for. However, we fully concur with Neggers et al. that additional markers beyond IGF-1 are required to assess residual disease activity in patients with acromegaly (Neggers et al., 2012).

It is an obvious limitation of our study that the number of patients with a suspicion for small tumour remnants and questionable residual disease activity despite initial GH suppression <1 ng/ml is quite small, so that a meaningful statistical evaluation has not been feasible so far. Thus, future studies (of larger size and with longer follow-up) will need to investigate whether the d3-GHR really is clinically relevant for follow-up and management decisions in patients with acromegaly. In addition, we propose that sKlotho may be a useful marker for the long-term follow-up of patients with acromegaly, as it seems to reflect the activity of GH-producing tumours, and we hope this will be tested in more detail in a larger number of patients.

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